

³²P primer for 141 Vent
Human spleen DNA

Project No. _____
Exhibit L-2
Appl. No. 09/558,421
B ok N . _____

67

ig N — ³²P 2633 (into the anchor primer)
follow P. 53 except use more ³²P ATP

~26% primers have ATP in 100% efficiency in labeling

ig 2633 159 μ M	1' μ l	✓	✓	✓	(159 μ M primer)	✓
³² P γ ATP 6000 Ci/mmol	2.5' μ l	✓	✓	✓	(41.8 μ M ATP)	✓
10 mCi/ μ l 10-21-94	6.25	✓	✓	✓		
(1.67 μ M ATP)	0.25 μ l	✓				
5X Kinase buffer	33.75					
PNK 50' μ l						

Any down 1126 ladder
10 μ l H₂O
1 μ l 34P dTP
15' 37°C
1 μ l EDTA

37°C 30 min → 5' 55°C → add

spin col same as P154, 7, and 145, 3

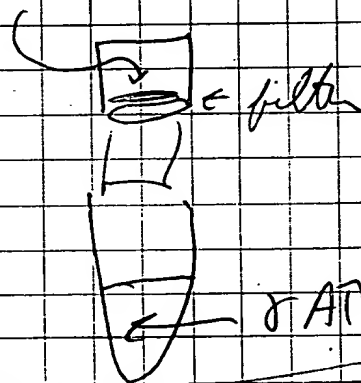
dilute ³²P 2633 with 100 μ l H₂O (Vp = 133 now)

spin in microfuge in "micron 3"

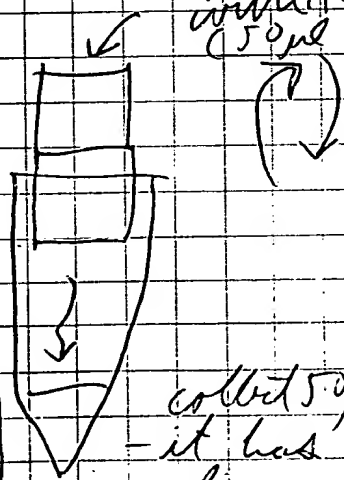
(amicon # 42402) - after all venting, put

add 200 μ l more H₂O and spin again

remove volume that did not enter filter



invert filter



10-24-94

Had a problem: filter kept peeling back on micron 3. Maybe g force was too high on Beckman microfuge "E" model will skip separation of free ATP.

³²P 2633 is diluted only 33.75 fold for Cf = 4.71 μ M

Issued & Understood by me, Researcher Pokany	Date 10/24/94	Invented by 	Date 10-19-94 10/24/94
		Recorded by	